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Size of the foveal blue scotoma related to the shape of the foveal pit but not to macular pigment

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ABSTRACT

When the eye is covered with a filter that transmits light below 480 nm and a blue field is observed on a computer screen that is modulated in brightness at about 1 Hz, the fovea is perceived as small irregular dark spot. It was proposed that the "foveal blue scotoma" results from the lack of S-cones in the foveal center. The foveal blue scotoma is highly variable among subjects. Possible factors responsible for the variability include differences in S-cone distribution, in foveal shape, and in macular pigment distribution. Nine young adult subjects were instructed to draw their foveal blue scotomas on a clear foil that was attached in front of the computer screen. The geometry of their foveal pit was measured in OCT images in two dimensions. Macular pigment distribution was measured in fundus camera images. Finally, blue scotomas were compared with Maxwell's spot which was visualized with a dichroic filter and is commonly assumed to reflect the macular pigment distribution. The diameters of the foveal blue scotomas varied from 15.8 to 76.4 arcmin in the right eyes and 15.5 to 84.7 arcmin in the left and were highly correlated in both eyes. It was found that the steeper the foveal slopes and the narrower the foveal pit, the larger the foveal blue scotoma. There was no correlation between foveal blue scotoma and macular pigment distribution or Maxwell's spot. The results are therefore in line with the assumption that the foveal blue scotoma is a consequence of the lack of S-cones in the foveal center. Unlike the foveal blue scotoma, Maxwell's spot is based on macular pigment as previously proposed.

1. Introduction

In 1894, Arthur König presented a lecture to the "Preussische Akademie der Wissenschaften" at Berlin, in which he claimed that the human fovea is "blue blind" and that subjects are "dichromatic" in the fovea (p. 591). His conclusion was based on psychophysical studies in which subjects had to fixate small monochromatic light spots presented at different wavelengths. He found that subjects had difficulties to distinguish between "blue" and "green" (König, 1894). Later, histological (Willmer & Wright, 1945) and psychophysical studies (Wald, 1967) confirmed that there is a tritanopic zone of about 20 arcmin in diameter in the center of the fovea (Williams, MacLeod, & Hayhoe, 1981). More recently, Curcio et al. (1991) mapped the foveal photoreceptors and found that a 20–25 arcmin S-cone free zone exists in the human foveola with sparsely and irregularly distributed S-cones in the adjacent foveal slopes. Under normal viewing conditions, the foveal blue scotoma is not visible because of the neural process of filling-in (Gerrits & Vendrik, 1970; Magnussen et al., 2001, 2004; Spillmann & Werner, 1996; Williams, MacLeod, & Hayhoe, 1981). However, Magnussen et al. (2001, 2004) described two procedures to make the blue scotoma visible. In the first study (Magnussen et al., 2001), subjects were presented with a blue field in Maxwellian view with a peak wavelength around 450 nm that was sinusoidally modulated in luminance at a frequency of 1–2 Hz. In this case, subjects could see their blue scotomas as a small dark spot that moved with their point of fixation. Apparently, the process of filling-in was compromised by the brightness modulation of the blue field. When subjects were asked to rate the visibility of the blue scotoma at different wavelengths, their ratings matched about the spectral sensitivity of the S-cones. In their second approach, Magnussen et al. (2004) showed that the foveal blue scotoma becomes visible as a bright spot in a negative afterimage when subjects were adapted to a bright blue field. Again, the subjects' rating as to how clearly they could see the blue scotomas varied with the peak wavelength of the adapting field and followed the spectral sensitivity function of the S-cones. The diameters of the perceived blue scotomas ranged from 24.8 to 44.3 arcmin, similar to the diameter of the S-cone free zone that was histologically identified in the foveal center by (Curcio et al., 1991).

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The primate fovea is histologically recognized as a pit with tightly packed M- and L-cones, providing maximal visual acuity. In this area, one single cone (M- or L-) is connected to 3–4 bipolar cells and 3 ganglion cells. This ratio decreases to one ganglion cell per cone at an eccentricity of 15–20 deg (3–4 mm). In peripheral retina there are more cones than ganglion cells. The ganglion cell density changes by a factor of 1000–4000 between peripheral and central retina (Wässle & Boycott, 1991; Wässle et al., 1990). Excluding S-cones from the foveal center appears to be an elegant trick to cope with chromatic defocus that results from longitudinal chromatic aberration of the optics of the eye (Rodieck, 1973). Shapes of foveas can be divided into two extremes: 'convexiclivate' and 'concaviclivate' (Polyak, 1951). Since the retinal tissue has a substantially higher refractive index than the vitreous (1.38 vs 1.335), the vitreo-retinal interface acts as a refracting surface. In a convexiclivate fovea, the interface acts as a magnifying glass to the image projected on the photoreceptors on the back of the retina. This design is found in reptiles, birds and some fishes. Harkness and Bennet-Clark (1978) have simulated the optical effects of the deep convexiclivate fovea and found that the perceived image distortions vary with the focus of the eye and could therefore be used as "focus indicator". On the other hand, in a concaviclivate fovea the image is minified because a flatter fovea is combined with a photoreceptor layer that is bulged out towards the center of the fovea pit, generating the effects of a concave lens. This case is mostly found in primates (Harkness & Bennet-Clark, 1978) but it is not clear what the advantage might be of minifying the projected image. The minification effect appears very small (<1%, see Section 4).

Interestingly, the shape of the foveal pit in human subjects is highly variable (see, for instance, OCT data in the current study) but probably not random since a negative correlation was found between the steepness of the foveal slopes and foveal diameter (Knighton & Gregori, 2012).

In the central region of the human retina, a yellowish macular pigment, consisting of lutein and zeaxanthin, is embedded in the cone axons and in the inner-plexiform layer. It acts as a screening pigment for the underlying photoreceptors (Hammond, Wooten, & Snodderly, 1997; Werner, Bieber, & Schefrin, 2000; Werner, Donnelly, & Kliegl, 1987) and is assumed to protect photoreceptors from photo-oxidative damage by short wavelength light (Kirschfeld, 1982; Nussbaum, Pruett, & Delori, 1981; Werner, Bieber, & Schefrin, 2000). The peak absorption of the macular pigment is around 460 nm (Bone, Landrum, & Cains, 1992), close to the spectral sensitivity peak of the S-cones (Stockman & Sharpe, 2000). The distribution of macular pigment varies considerably among subjects (Wooten & Hammond, 2002; Wooten et al., 1999). It is assumed that the percept of Maxwell's spot is related to the macular pigment distribution.

Since foveal shape, foveal blue scotomas, and macular pigment distribution are all highly variable among subjects, it is interesting to study how they are related. Furthermore, there is recently increasing interest in this question (i.e. the ongoing MacTel project https://web.emmes.com/study/mactel/). To further elucidate the relationship between macular pigment distribution and foveal blue scotoma, we also explored how they are related to the appearance of Maxwell's spot.

2. Methods

2.1. Subjects

Nine subjects (5 female and 4 male) with an average age of 29.6 ± 7.7 years (ranging from 22 to 49 years) and normal color vision were recruited for the experiments. The Chinese subjects (1, 3, 5, 8) had undergone color vision testing with the Ishihara pseudo-isochromatic color plates prior to their enrollment at their home universities. The remaining German subjects were tested at school and had no known color vision deficiencies. The study adhered to Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the local University Ethics Commission.

2.2. Psychophysical experiments

2.2.1. Measurement of the foveal blue scotomas

A blue field (size 900 × 900 pixel), sinusoidally modulated in luminance at a frequency of 1 Hz between RGB (0, 0, 255) and RGB (0, 0, 0), was presented on a thin film transistor (TFT) display (screen refresh rate 60 Hz, EIZO FlexScan S1921, 19 in.). Maximal pixel radiance of the "B" channel (RGB (0, 0, 255)), as measured by a photometer (Minolta LS100), was 10.70 cd/m^2. Since the "blue" gun of computer screen contains energy also in the middle wavelength range, M- and L-cones were also stimulated by the B gun. To preferentially stimulate the S-cones, a filter excluding light above 500 nm was needed. We used the bandpass glass filter BG25 (Schott, Germany) with a peak transmission at about 400 nm and a FWHM of about 50 nm. Subjects viewed the modulated "blue" field on the screen in a dark room from a distance of 74 cm. The blue field had a diameter of 26.4 × 26.4 cm on the screen which converts into a visual angle of 20.2 deg. Assuming a retinal image magnification for the human eye of 290 μm/deg (Gullstrand, 1909), the linear size of the retinal image was about 5850 μm.

Subjects were instructed to draw their foveal blue scotomas, one eye after the other, with a marker pen on a transparent plastic sheet that was attached in front of the screen. The procedure was repeated four times. The four drawings were averaged pixel by pixel using "ImageJ" (http://rsb.info.nih.gov/ij/). Image J offers an "image calculator" function which calculates the arithmetic mean of each pixel gray value from two or more images. The resulting "average" of several drawings made it more easy to measure the diameters of the foveal blue scotomas, or of Maxwell's spots, and also increased the confidence in the measurements.

2.2.2. Visualization and measurement of Maxwell's spot

Maxwell's spot was visualized as described by Isobe and Motokawa (1955). A bright white field was generated by aiming a video projector (Sharpe XG-NV21SE) at a white paper that was attached to the wall. Subjects were instructed to look into the bright white field through a dichroic filter in front of one eye, the other eye was covered (KIF 483, Schott, Germany; light transmission below 480 nm and above 610 nm, with prominent attenuation between 500 and 600 nm). Typically, Maxwell's spot becomes visible as a brownish or reddish spot on bright white background with variable shapes and diameters among different subjects, as shown in Fig. 6. Transmission of the dichroic filter also at longer wavelengths is necessary and was already recommended by Maxwell himself to counteract retinal adaptation. When subjects look into white light without a filter, Maxwell's spot disappears almost immediately due to rapid adaptation of macular photoreceptors (Miles, 1954). In order to maintain visibility of Maxwell's spot, a dichromatic filter was also used by Holm (1922), Walls and Mathews (1952), and Isobe and Motokawa (1955). As in the previous experiments where the blue scotoma was measured, the distance between the subject and the wall was 74 cm. The instructions to the subjects were to draw the pattern that they saw directly on the paper. It was mentioned to them that, while the blue scotoma appears as a dark gray or black spot, Maxwell's spot looks reddish or brownish.

2.3. Optical Coherence Tomography (OCT)

Foveal shape was measured in the horizontal and vertical direction by a high-resolution OCT (Spectralis HRA + OCT, Heidelberg Engineering, Heidelberg, Germany) in the 9 subjects (18 eyes). 512 A-scans were performed in each B-scan with a scan length of 20 deg in terms of visual angle (linear distance on the retina approximately 6 mm). The minimal foveal thickness (MFT) was assumed to define the center of the fovea. It was automatically determined by built-in software of the instrument. Differences in retinal thickness (ΔRT) were determined between the center of the fovea and at four parafoveal positions (0.4 and 0.8 mm away from the central fovea on either side) using ImageJ. ΔRTs were plotted against the dimensions of the drawn foveal blue scotomas. The diameter of the floor of the central fovea was also measured, defined as the area where retinal thickness remained at a minimum. While different OCT devices may select different boundaries for measuring retinal thickness (Giani et al., 2010), we were interested in inter-individual differences. Such differences would show up no matter which boundary is used, as long as the device and the criteria are always the same.

2.4. Fundus photography and measurement of the macular pigment distributions

Distributions of the macular pigment were analyzed in fundus pictures in each eye of the nine subjects, obtained by a Non-Mydriatic Retinal Camera (Nonmyd WX3D, Kowa, Germany). The fundus pictures were taken in the University Eye Hospital Tuebingen and the responsible ophthalmologist confirmed that no pathological features were apparent. For analysis, the RGB fundus pictures were separated into their 3 RGB channels, again using "ImageJ". Since the macular pigment has apeak absorption around 460 (Bone, Landrum, & Cains, 1992), the analysis was done in the image generated by the blue channel of the RGB format. "ImageJ" provides a function to measure the number of pixel that are above an adjustable threshold. A square of 4 × 4 deg (244 × 244 pixels) was adjusted to cover the macular area (gray box in Fig. 1A). The threshold was adjusted to 50% of the average brightness of the pixels in the square. Supra-threshold pixels were marked in gray (Fig. 1B) and the horizontal diameter of the marked area was measured in pixels. Conversion into visual angle was simple since one pixel in the fundus image was equivalent to about one arcmin. Our procedure produced diameters of the pigmented areas (Table 3; average diameter 2.36 deg, radius 1.18 deg) that are comparable to those in the literature. Hammond, Wooten, and Snodderly (1997)described a mean radius of the distribution at half maximum in macular pigment optical density (MPOD) of 1.03 deg, Makridaki et al. (2009) 1.30 deg and Smith et al. (2004) 1.03 deg, similar to the data in the current study (1.18 deg). Furthermore, the macular pigment densities were quantitatively analyzed using their pixel brightness profiles (Fig. 1C), with the profiles again provided by ImageJ.

2.5. Statistical analysis

Linear regression and correlation analysis was performed in Microsoft Excel (Asknet AG, Germany). Significance was assumed at $p < 0.05$.

3. Results

3.1. Shapes and diameters of the perceived blue scotomas

The shapes of the perceived foveal blue scotomas, as determined from the drawings of the subjects, are shown in Fig. 2. Reproducibility was high which can be seen when the individual drawings are compared to the averages of four repetitions. Two types of blue scotomas were observed, a small spot (type 1: subjects 2, 4, 5, and 9) and a circular shape in the center, surrounded by star-shaped radial extensions (type 2: subjects 1, 3, 6, 7 and 8).

Horizontal and vertical diameters of the perceived blue scotomas are listed in Table 1. In the right eyes, visual angles ranged from 15.75 to 76.35 arcmin (3.4 to 16.4 mm on the computer screen at 74 cm distance). In left eyes, they ranged from 15.50 to 84.72 arcmin.

Significant correlations were found between both eyes, both in the horizontal and vertical diameters of blue scotomas (Fig. 3, horizontal $R = 0.969$, df = 8, $p < 0.01$; vertical $R = 0.976$, df = 9, $p < 0.01$).

3.2. Optical Coherence Tomography (OCT) of the foveal region

The minimum values for the foveal thickness (MFT, the location of the central fovea) and dimensions of the bottom diameter of the foveal pit for each subject are listed in Table 2. The average MFT was 0.253 ± 0.023 mm in the right eyes, and 0.257 ± 0.039 mm in the left. The diameter of the floor of the foveal pit in the right eyes was on average 0.12 ± 0.04 mm (24.8 arcmin) wide, and 0.15 ± 0.05 mm (31.0 arcmin) in the left eye (Table 2).

To quantify the slopes of the foveal pit, two lateral positions from the foveal center were chosen, ±0.4 mm, and ±0.8 mm. The difference of retinal thickness (ΔRT) between the central fovea

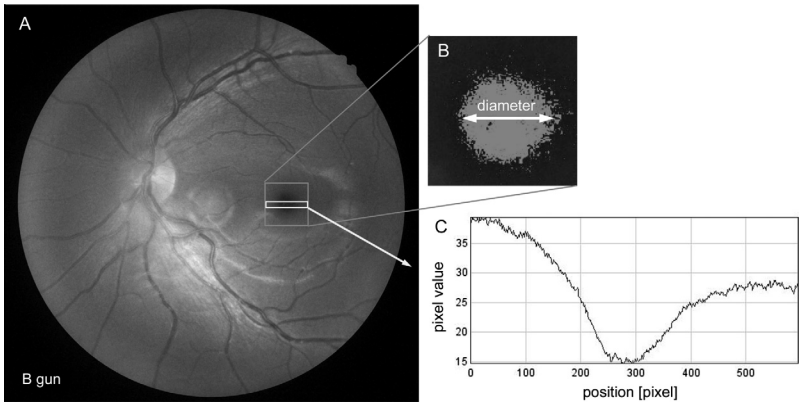

Fig. 1. (A) Fundus picture generated from the B gun of the RGB format. The gray box denotes a square of 244 × 244 pixel in which pixels were thresholded and extracted as shown in (B) to measure the diameter of the macular pigment distribution (white arrow). In (C), the pixel brightness profile is shown as extracted from the pixels in the white rectangle, denoting the macular pigment optical density (MPOD). One pixel is equivalent to about 1 min of arc.

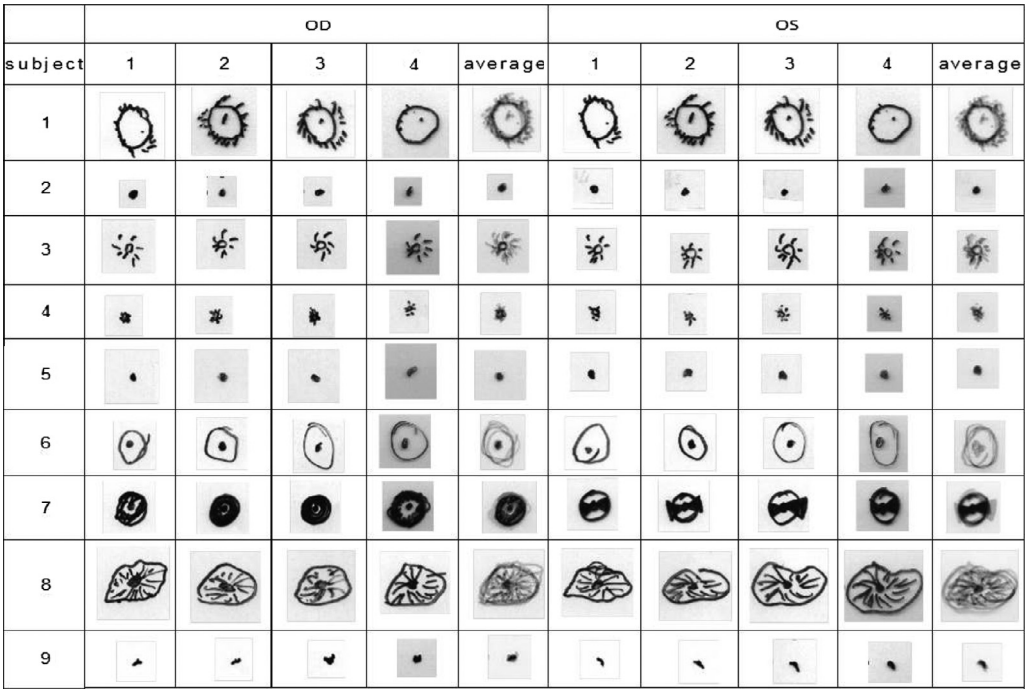

Fig. 2. Original drawings by the subjects of their blue scotomas. Four repetitions are shown, followed by their averages, as determined by "ImageJ".

Table 1
Diameters of the perceived foveal blue scotomas in nine subjects, as determined from averages of four drawings prepared by each subject.

Subject	Right eyes (OD)		Left eyes (OS)	
	Vertical visual angle (arcmin)	Horizontal visual angle (arcmin)	Vertical visual angle (arcmin)	Horizontal visual angle (arcmin)
1	77.9	76.4	89.7	84.7
2	16.4	16.3	13.7	15.8
3	50.8	49.4	50.2	45.2
4	24.3	21.7	25.0	25.1
5	14.9	15.8	17.5	17.0
6	71.5	65.0	74.0	64.5
7	61.8	63.2	57.6	60.8
8	48.5	67.1	36.9	60.8
9	17.1	20.3	16.4	15.5

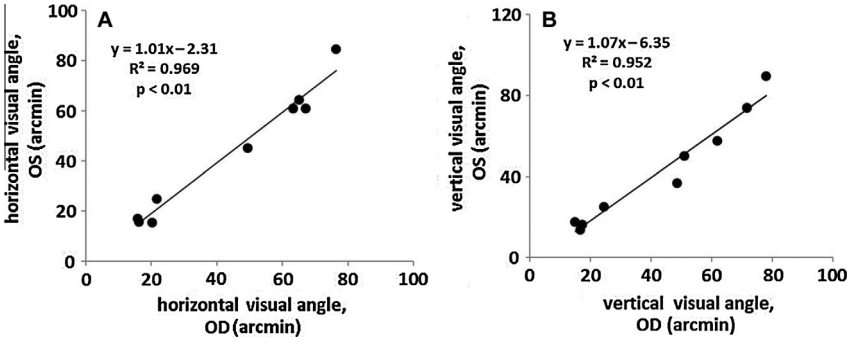

Fig. 3. Correlations of the diameters of the perceived blue scotomas between the left and right eyes of the nine subjects, both in horizontal (A) and vertical directions (B). OS = left eyes, OD = right eyes.

and four parafoveal positions was measured by "ImageJ" in pixels as shown in Fig. 4. One pixel was equivalent to 1.90 μm. Significant correlations were found between ΔRT values on both sides of the foveal center (OD: 0.4 mm vs −0.4 mm: $R = 0.64$, df = 8, $p < 0.05$; 0.8 mm vs −0.8 mm: $R = 0.922$, df = 8, $p < 0.01$; 1.2 mm vs −1.2 mm: $R = 0.963$, df = 8, $p < 0.01$. OS: $R = 0.814$, 0.919, 0.965 for each pair, df = 8, $p < 0.01$).

3.3. Correlations of the diameters of the foveal blue scotomas and foveal geometry

Significant correlations were found between the diameters of the foveal blue scotomas and the increase in retinal thickness at ±0.4 mm and ±0.8 mm from the foveal center (R ranging from 0.634 to 0.723 in the right eyes, $p < 0.05$ in all cases, df = 8, shown

Table 2
Minimum foveal thickness (MFT, the minimum thickness of the retina in the center of the fovea) and diameter of the floor of the foveal pitas measured by built-in software of the "Heidelberg Eye Explorer". OS = left eyes, OD = right eyes.

Subject	MFT (mm)		Diameter of the floor of foveal pit (arcmin)	
	OD	OS	OD	OS
1	0.272	0.246	11.57	26.62
2	0.263	0.207	27.78	28.93
3	0.272	0.266	12.73	40.51
4	0.250	0.250	32.41	35.88
5	0.281	0.344	32.41	40.51
6	0.225	0.241	28.93	27.78
7	0.231	0.220	24.30	25.46
8	0.218	0.260	20.83	13.89
9	0.269	0.275	26.62	43.98

in Fig. 5, and R ranging from 0.651 to 0.741, in the left; plot of left eye data not shown). The correlations were always positive indicating that the steeper the foveal slope, the larger the foveal blue scotoma. Similarly, the diameters of the foveal blue scotomas were correlated to the diameters of foveal pit (OD: $R = -0.656$, df = 8, $p < 0.05$; OS: $R = -0.645$, df = 8, $p < 0.05$; shown in Fig. 5). The negative correlations indicate that a wide foveal pit was associated with a small foveal blue scotoma while a narrow foveal pit was associated with a large blue scotoma. We also analyzed the relationship between the steepness of the foveal slopes (from 0 to 0.4 mm and from 0 to 0.8 mm) and the horizontal diameters of the foveal blue scotoma. Correlations were similarly significant ($p < 0.05$).

At 1.2 mm distance from the foveal center, no significant correlations were found between the increase in retinal thickness and the blue scotoma. In several subjects, ±1.2 mm was already outside the foveal pit.

3.4. Macular pigment distributions and perceived blue scotomas

The horizontal diameters of the macular pigment distributions are shown in Table 3. Repeatability of measurement was tested by correlating the data gathered by two independent observers (observer 1 vs observer 2: OD: $R = 0.913$, df = 8, $p < 0.01$; OS: $R = 0.823$, df = 8, $p < 0.01$). The average horizontal diameters of the macular pigment distributions were 0.69 ± 0.19 mm (2.37 deg) in the right eyes and 0.68 ± 0.18 mm (2.34 deg) in left (note that the 50% criterion for detection of the borders of the pigmentation may underestimate the total width of the distributions). With this criterion, subject 1 had the smallest pigmented area (horizontal diameter right eye, 0.32 mm; left eye 0.29 mm) and subject 8 the largest (right eye 0.96 mm, left eye 0.95 mm). The diameters of the macular pigment distributions were highly correlated in left and right eyes ($R = 0.929$, $p < 0.01$). However, no correlation was found between the extension of the pigmented area and the diameters of the perceived foveal blue scotomas ($R < 0.179$ in all cases, df = 8, n.s.).

3.5. Possible contributions of the macular pigment to the foveal blue scotoma

Above, the margins of the area covered with macular pigment were defined by a 50% brightness criterion for the pixels in the "blue" channel in the RGB images of the fundus. However, a 50% threshold criterion does not provide the full information about the macular pigment distribution. Therefore, we plotted the density of the macular pigment, as inferred from the pixel values in the "blue" channel in the horizontal meridian and compared it to the diameters of the foveal blue scotomas (Fig. 6). It is obvious that the angular diameters of the blue scotomas were smaller than of the macular pigment distributions in most subjects. Magnussen et al. (2004) also compared the size of the blue scotomas with Haidinger's brushes, an entoptic phenomenon that occurs due to the dichroic properties of macular pigment. They also concluded that macular pigment is unlikely to contribute to the foveal blue scotoma. It was previously found that the slope of the foveal pit is unrelated to the macular pigment distribution (Westrup et al., 2014).

Diameters of the Maxwell's spots as perceived by the 8 subjects are shown in Table 4.

3.6. Foveal blue scotoma and Maxwell's spot

Maxwell's spot is described in the literature as "a darker ring or shell-burst" (Miles, 1954), "a spot rounded by a clear ring and a halo" (Isobe & Motokawa, 1955), or as a "dark diffuse spot", a "dark spot with star", a "dark ring with no central dark spot" or a "dark ring with central dark spot". In our study, 8 of the 9 subjects were available and were instructed to look at bright white field through the dichroic filter. Subjects were asked to draw their perceived Maxwell's spot (Fig. 5). One subject (subject 4) could not see Maxwell's spot at all, subjects 2 and 5 saw it only with one eye. The drawings of the remaining subjects had little similarity with their drawings of the blue scotomas: (1) Maxwell's spot was always larger (average: 105 ± 35 arcmin) than the blue scotomas, (2) Maxwell's spot was more blurry and weaker, and (3) Maxwell's spot appeared as a reddish or brownish spot. When left and right eyes were treated as independent samples, the diameters of the perceived Maxwell's spot were correlated to the diameters of the macular pigment distributions (Fig. 7, $R = 0.563$, df = 11, $p < 0.05$). Therefore, we concur with Delori et al. (2006) who state that "Maxwell's spot matched the measured macular pigment distributions".

4. Discussion

We found that the diameters of the foveal blue scotomas were highly variable among subjects but closely correlated in both eyes. We also found that the diameter of the foveal blue scotomas were related to the steepness of the foveal slope – the steeper the

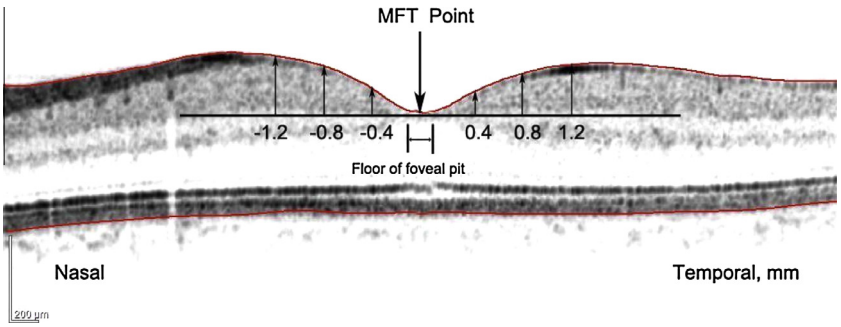

Fig. 4. OCT scan of the foveal area as provided by the "Heidelberg Eye Explorer", indicating the foveal center (MFT). Vertical black lines indicate the six eccentricities where retinal thickness was determined and correlated to the diameters of the perceived blue scotomas.

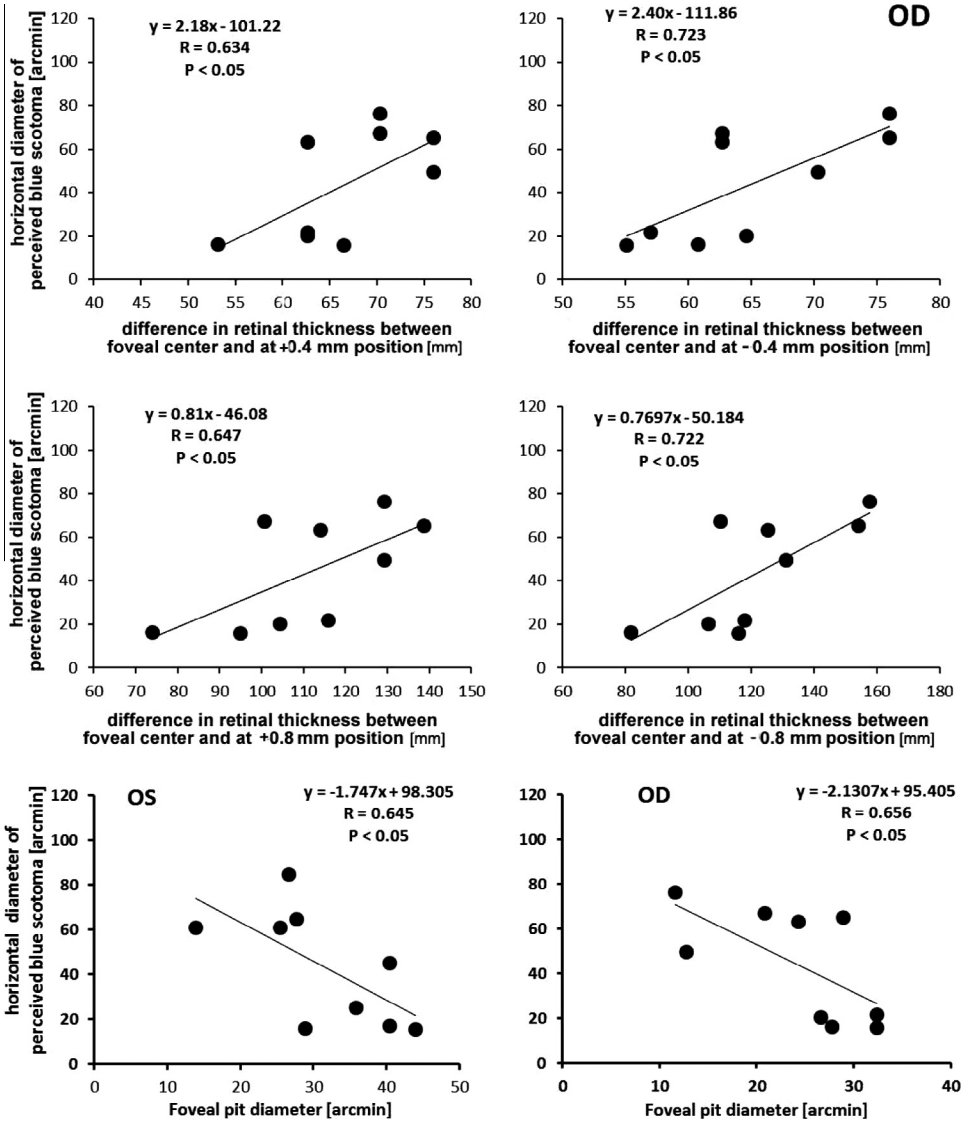

Fig. 5. Horizontal diameters of the perceived foveal blue scotomas plotted against the increase in retinal thickness from the foveal center in the right eyes (OD) of the nine subjects (top four figures). In the lowest two figures, the horizontal diameters of the perceived foveal blue scotomas are plotted against the diameters of the floor of the foveal pit. OS = left eyes, OD = right eyes.

Table 3
Horizontal diameters of the macular pigment distributions in the nine subjects, using a criterion of a drop in brightness below 50% in the RGB blue channel, provided in mm and arcmin. The diameters of the macular pigment distributions were highly correlated in left and right eyes ($R = 0.929$, $p < 0.001$). OS = left eyes, OD = right eyes.

Subject	OD		OS	
	Diameter of pigmented areas (mm)	Diameter of pigmented areas (arcmin)	Diameter of pigmented areas (mm)	Diameter of pigmented areas (arcmin)
1	0.32	65.5	0.29	59.5
2	0.50	103.2	0.65	134.9
3	0.86	178.6	0.79	162.7
4	0.78	160.7	0.75	154.8
5	0.64	131.9	0.61	127.0
6	0.64	131.9	0.67	138.9
7	0.77	158.7	0.81	166.7
8	0.96	198.4	0.95	196.4
9	0.72	147.8	0.63	130.9

increase in retinal thickness from the foveal center to the foveal rim, the larger the blue scotoma. These findings indicate that the blue cone distributions in the fovea interact with the shape of the fovea although it is not known how and at which time during development this happens. We also found that the macular pigment distributions were highly correlated in both eyes but cannot account for the inter-individual differences in the size of the foveal blue scotomas.

4.1. Appearance and diameters of the S-cone free zone as described in the literature

The drawings of the blue scotomas as perceived by our subjects match descriptions in the literature: "the scotoma appears as a small, colorless dark spot with irregular, ragged borders in central vision" (Magnussen et al., 2001). They also match the patterns of the S-cone free zone described by Curcio and colleagues, and Williams and colleagues (Curcio et al., 1991; Williams, MacLeod, & Hayhoe, 1981). Also the diameters are in close agreement with the literature. The S-cone free zone was described as "about 20–25 arcmin wide, surrounded by an irregular pattern of S-cones at the foveal rim" (Curcio et al., 1991), 24–32 arcmin (Magnussen

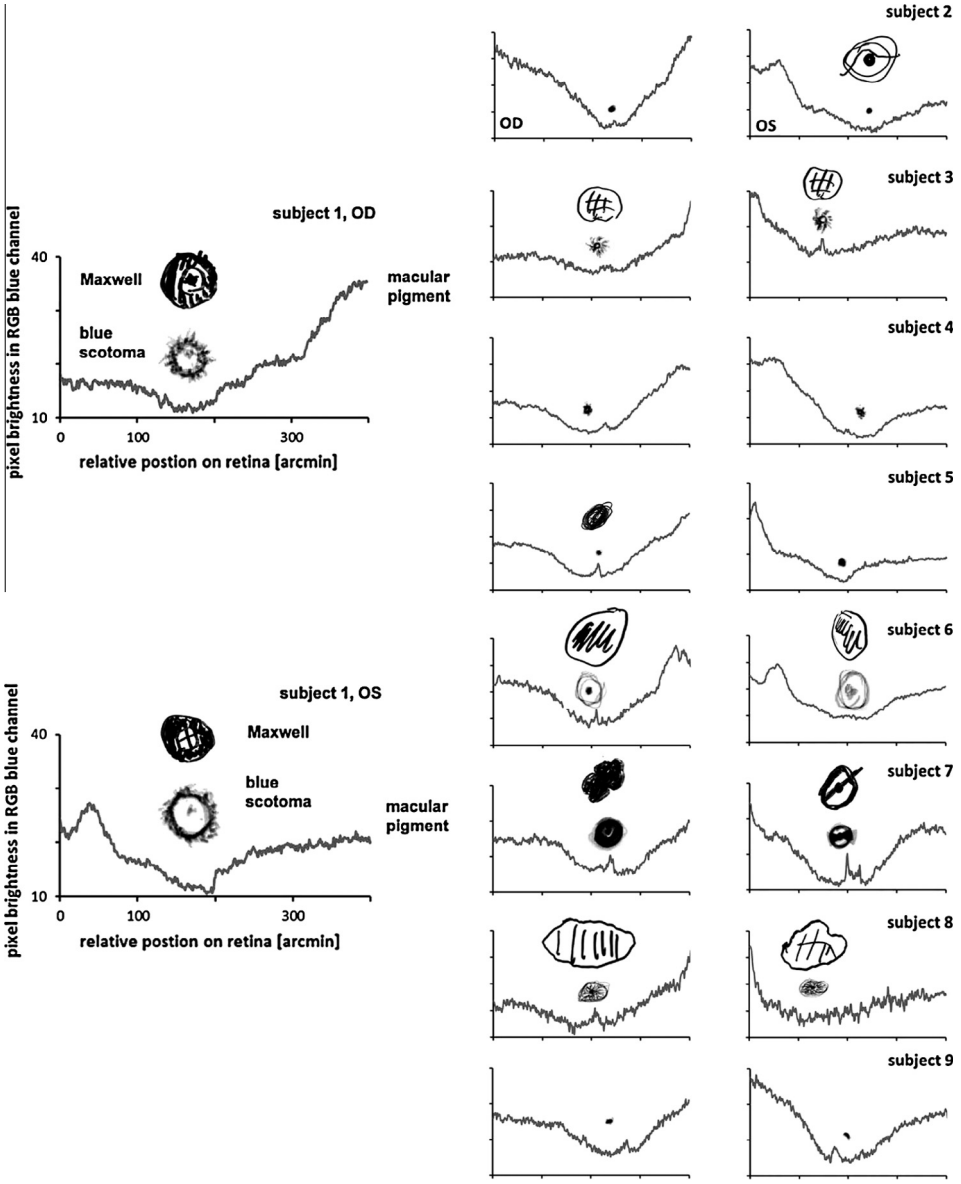

Fig. 6. Macular pigment density profiles in the horizontal meridian, as measured in 9 subjects. Drawings of the Maxwell's spot and foveal blue scotomas are also shown, adjusted to the same angular magnification (1 pixel on the fundus = 1 arcmin). Subject 9 was no longer available for the measurements of Maxwell's spot. Subject 4 could not see Maxwell's spot at all. Subject 2 and subject 5 saw it only in one eye. Note the large differences in perceived size between foveal blue scotoma and Maxwell's spot in subjects 2, 3, 5, 7 and 8. OS = left eyes, OD = right eyes.

Table 4
Horizontal diameters of Maxwell's spots as measured in visual angles (arcmin). OS = left eyes, OD = right eyes.

	OD	OS
1	67.2	80.7
2	No	130.6
3	95.7	102.8
4	Not seen	Not seen
5	65.1	Not seen
6	132.3	73.5
7	92.0	95.2
8	191.9	134.8

et al., 2001), or 24.8–44.3 arcmin (Magnussen et al., 2004). In our study, the drawings showed that the central scotomas were sometimes surrounded by an irregular circle. In these cases, the total diameters of area of the blue scotomas were larger than previously described. The dimensions of central spot still matched the literature data (Magnussen et al., 2001, 2004). The linear diameter of the human fovea was described as 600–750 μm (2.0–2.5 degree of visual angle; Yuodelis & Hendrickson, 1986). The foveal avascular zone diameter (FAZ) in recent studies ranges from 0.20 to 1.08 mm (Dubis et al., 2012). Obviously, the S-cone free zone covers only a small proportion of the center of the foveal pit.

4.2. Relations of foveal shape and blue scotomas to gender and age

Delori et al. (2006) found that women have broader macular pigment distributions than men. They also found that "the radius of curvature of the concave inner limiting membrane surface is ... larger in women than in men (1185 and 744 μm, respectively), which is consistent with a flatter foveal floor and/or broader foveal depression". In our study, we did not see a correlation of foveal shape or diameter of the blue scotomas with gender

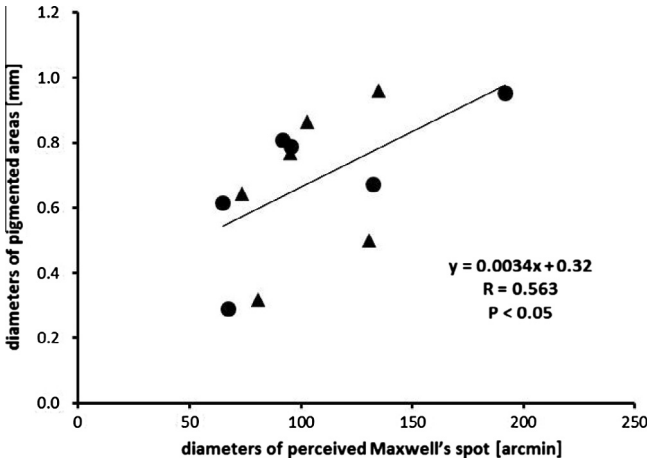

Fig. 7. Correlation between Maxwell's spot and diameter of macular pigment distributions. Triangles represent data from left eyes and circles from right eyes.

(5 male and 4 females were tested). It is possible that more than 9 subjects are necessary to confirm such a relationship.

Recently, it was reported that the area covered with macular pigment increases with age (Baptista & Nascimento, 2014). If macular pigment played a role in the percept of the foveal blue scotoma, there should have been a change with age. However, no significant correlation with age was found in our sample, may be, because their ages were too similar and the sample size too small ($R < 0.225$, df = 8, $p > 0.05$ in either eye).

4.3. Current conventions in the evaluation of the morphology of the fovea

The group of Joe Caroll (Dubis et al., 2012; Wagner-Schuman et al., 2011) used the change of the foveal slope to define the central fovea, the foveal pit depth and the foveal rim. In our measurements, we basically used the same measurement parameters:

(1) We defined the center of the fovea as the area where the retina was thinnest. In the slope method, the center was defined as the area where the slope was zero (Dubis et al., 2012) or its value switched from negative to positive (Wagner-Schuman et al., 2011).
(2) While we did not measure foveal depth as "the axial distance between a plane connecting the foveal rims and bottom of the foveal pit", the differences in retinal thickness (ΔRT) in the six positions evaluated in our study were measured in a similar way. We used the transversal distance between the bottom of the foveal pit and four parafoveal positions.
(3) While these data are not shown in the manuscript, we used the similar way to locate the foveal rim. On either side of foveal center, the foveal rim was located as the position where the retina reached the first peak thickness which is equivalent to the position where the slope is zero. A difference is the foveal profile was measured at 5 different angular positions, 30 deg away from each other, while we measured only in the horizontal meridian (Dubis et al., 2012; Wagner-Schuman et al., 2011) while we measured only in the horizontal and vertical meridians.

Our measurement results were similar to the ones by Wagner-Schuman et al. (2011) and Dubis et al. (2012). We also found that, at 0.8 mm distance from the foveal center, ΔRTvalues are significantly correlated in both eyes ($R = 0.9621$ and 0.8854, df = 8, $p < 0.01$). Center foveal thickness was 0.20–0.32 mm in their study, and 0.22–0.34 mm in ours. The slopes of the foveal pit were also significantly correlated between both eyes in their study, as well as in ours. We also did not find any gender bias, similar to Wagner-Schuman et al. (2011). We did not show data on the foveal rim diameters in our manuscript but these data were similar to the ones by Wagner-Schuman et al. (2011) (1.5–2.5 mm vs 1.74–2.76 mm in our study). Two other variables (central retina thickness in two eyes, gender bias in retinal thickness in the central fovea) did not produce any significant correlations in our study although they were in the same absolute range, probably because our sample size was much smaller ($n = 9$ subject, 18 eyes, versus $n = 90$ subject, 180 eyes). We did not analyze other parameters like foveal pit diameter, foveal pit volume and foveal pit area.

4.4. Comparison to other studies on the diameters of the macular pigment distributions

Westrup et al. (2014) also studied the relationship between foveal geometry and macular pigment distribution. They found that interocular correlations for several measures of retinal thickness (RT) and RT layers were high. RT was inversely related to MPOD at 1 and 2 deg from the foveal center, but not to central MPOD. The radius of the pigmented areas came out similar (their half-width 1.19 deg vs 1.18 deg in our study). A difference was that they used the green channel image whereas we used the blue channel image.

4.5. Relationship between the blue scotoma and the geometry of the foveal pit

In the current study, 5 subjects reported extra star-shaped radial extensions outside the central dark spot had larger blue scotomas. These subjects also had steeper foveal slopes and smaller foveal pit diameters, as can be seen in Fig. 4. Apparently, the anatomical structure of the foveal pit interacts with the foveal blue scotoma and determines whether star-shaped radial extensions are seen. There are at least two mechanisms that could explain these findings: (1) flatter foveas should have less minification effect as they act as a negative lens which could cause smaller blue scotomas and (2) S-cones embedded in the foveal slopes could be more tilted with respect to the incoming rays which could reduce their sensitivity due to their Stiles–Crawford effect (Burns et al., 1997a, 1997b; Stiles & Crawford, 1933). However, both hypotheses suffer from shortcomings: (1) The estimated magnification effects of the foveal pit are very small and cannot account for the magnitude of the differences that were observed among the subjects. The estimated change in retinal image magnification, derived from the approximate radius of the fovea (about 4.3 mm, calculated from foveal width of 2 mm and a depth of 0.12 mm) and the refractive indices of vitreous and retina ($n = 1.335$ and $n = 1.380$) were only about 0.22%. (2) While the perceived blue scotomas were often wider than the diameters of the foveal floor (foveal floor widths ranging from 19.7 to 26.9 arcmin (Table 2), blue scotomas ranging from 15.5 to 84.7 arcmin (Table 1)), it is highly unlikely that the S-cones located in the foveal slopes would have been so misaligned that they were almost not stimulated. Therefore, none of these explanations is convincing and further studies are required to uncover the underlying mechanisms.

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